Genetic and Molecular Analysis on Estrogen Receptor Polymorphisms

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ABSTRACT

Estrogen hormone exposure has been implicated in increasing the risk of breast cancer. Estrogen functions by binding to response elements found in a gene to further any downstream process. Genetic studies on Estrogen receptor binding sites had confirmed 7 SNPs to have a high risk in causing breast cancer. This study investigated whether one of those confirmed SNPs rs12440374 in the ER binding site plays a role in breast cancer risk either as a protective factor or as a risk factor. It was analysed that the allelic variant C of this SNP enhances the binding of estrogen to ERE thereby being a risk factor to breast cancer.

INTRODUCTION

Breast cancer is the most common cancer occurring in women accounting for about 20% of all female cancers. Various risk factors have been implicated for causing breast cancer, such as genetic constitution, ethnicity, gender, age of menarche and menopause etc. A positive family history of breast cancer is considered a well established risk factor for breast cancer. It was found that breast cancer occurrences are twice as common in women with first-degree relatives having the disease in comparison to the general population. Linkage and sequence analysis studies on patients with family history of cancer have indicated six to seven high risk breast cancer susceptibility genes which include BRCA1, BRCA2, PTEN, TP53, LKB1/STK11 and CDH1.

Although a lot is known about genes involved causing familial breast cancer, this accounts for only about 5-10% of the total breast cancer cases. Furthermore another study conducted on twin cohorts by Lichtenstein et.al in 2000 has shown that the contribution of heritable factors to breast cancer is only 27%. Another major risk factor identified to cause breast cancer is the exposure to sex hormones, estrogen in particular. The classical pathway of estrogen action involves the binding of estrogen receptor to Estrogen Binding Elements (ERE) which then activates other downstream process.

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Recent studies conducted have targeted the genome to analyze several Single Nucleotide Polymorphisms (SNPs) in association studies to identify risk alleles without having prior knowledge about their position or function. ChIP-Paired End diTag (ChIP-PET) study was conducted in MCF-7 cells and 1,234 high quality ERα binding sites were identified. The genetic study of these identified binding sites is being conducted currently in a large Swedish cohort of postmenopausal women. SNP analysis was done for common polymorphisms selection and 7 SNPs were confirmed to have a high risk factor for breast cancer. This study involves one of those confirmed SNPs rs12440374. The main aim of this study is to investigate whether rs12440374 polymorphism in the ER binding site plays a role in breast cancer risk by either as a protective factor or as a risk factor.

MATERIALS AND METHODS

Statistical analysis was conducted to confirm the significance of the SNP being studied. DNA samples were amplified for SNP and ERE region, ligated into PGL4-TATA vector and cloned into TOP10 cells. Plasmid DNA extracted was sequenced and analysed for the presence of SNP and ERE region. It was then transiently transfected into MCF-7 cells along with Renilla. Dual-Luciferase assay was conducted and the results analysed.

RESULTS AND DISCUSSION

Statistical analysis revealed a p. value of 0.027 which is considered significant for the chi-square test conducted. Recombinant cloning was successful and positive colonies were observed. Sequencing analysis confirmed the presence of ERE region and SNP. Two allelic variants of SNP was confirmed C/T. Transient transfection and drug treatment were conducted prior to Dual-Luciferase assay were successful. Dual-Luciferase Assay indicated that the allelic variant C of the SNP rs12440374 caused an enhanced binding of estrogen to ERE indicating that it poses as a risk factor for breast cancer occurrence.

CONCLUSION

In this study the functional analysis of the SNP rs12440374 in the estrogen receptor element in the intron of gene FLJ39743 was successfully carried out on ordered DNA samples. The allelic variant C of the SNP was found to have enhanced the Estrogen binding to ERE, thereby confirming that the presence of this variant increase breast cancer risk.
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UCSC Genome Browser, [http://genome.ucsc.edu](http://genome.ucsc.edu).